

### AMENDMENTS TO THE SPECIFICATION

In the specification at page 1, line 8, please replace the paragraph which starts with "Thus, for example" with the following amended paragraph:

Fungi of the genus Blakeslea are known as production organisms. Thus, for example, Blakeslea trispora is used as a producer organism for  $\beta$ -carotene (Ciegler, 1965, Adv Appl Microbiol. 7:1) and lycopene (EP 1201762, EP 1184464, WO 03/038064). In addition, Blakeslea is suitable for producing other lipophilic substances such as, for example, other carotenoids and their precursors, phospholipids, triacylglycerides, steroids, waxes, fat-soluble vitamins, provitamins and cofactors or for producing hydrophilic substances such as, for example, proteins, amino acids, nucleotides and water-soluble vitamins, provitamins and cofactors.

In the specification at page 30, line 28, please replace the paragraph which starts with "One example of a hydroxylase" with the following amended paragraph:

One example of a hydroxylase gene is a nucleic acid encoding a Haematococcus pluvialis hydroxylase, with accession No. AX038729 (WO 0061764; nucleic acid: SEQ ID NO: 31, protein: SEQ ID NO: 32), ~~an~~ from Erwinia uredovora 20D3 hydroxylase (ATCC 19321, accession No. D90087; nucleic acid: SEQ ID NO: 33, protein: SEQ ID NO: 34) or Thermus thermophilus hydroxylase (DE 102 34 126.5) encoded by the sequence SEQ ID NO 76.

In the specification at page 31, line 25, please replace the paragraph which starts with "In the preferred embodiment" with the following amended paragraph:

In the preferred embodiment described above, preference is given to using as hydroxylase genes nucleic acids which encode proteins comprising the amino acid sequence SEQ ID NO: 32, 34 or encoded by the sequence SEQ ID NO ~~NO~~ NO: 76 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of least 30%, preferably at least 50%, more preferably at least 70%, still more preferably at least 80%, ~~more~~ most preferably at least 90%, in particular 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99%, at the amino acid level to the sequence SEQ. ID. NO: 32, 34, or encoded by the sequence with SEQ ID ~~NO~~ NO: 76, and which have the enzymic property of a hydroxylase.

In the specification at page 37, line 15, please replace the paragraph which starts with "The *Blakeslea trispora*" with the following amended paragraph:

The *Blakeslea trispora* strains ATCC 14271 (mating type (+)) and ATCC14272 (~~+~~) ~~mating~~ (mating type (-)) were obtained from the American Type Culture Collection. *B. trispora* were grown in MEP medium (malt extract-peptone medium): 30 g/l malt extract (Difco), 3 g/l peptone (Soytone, Difco), 20 g/l agar, pH set to 5.5, ad 1000 ml with H<sub>2</sub>O at 28°C.

In the specification at page 40, line 26, please replace the paragraph which starts with "To select for transformed" with the following amended paragraph which continues onto page 41:

To select for transformed *Blakeslea* cells, the medium contained hygromycin at a concentration of 100 mg/l and, to select against agrobacteria, 100 mg/l cefotaxime. The incubation was carried out at 26°C for approx. 7 days. This was followed by transferring mycelium to fresh selection plates. Resultant spores were rinsed with 0.9% NaCl and plated on CM17-1 agar (3 g/l glucose, 200 mg/l L-asparagine, 50 mg/l MgSO<sub>4</sub> x 7H<sub>2</sub>O, 150 mg/l KH<sub>2</sub>PO<sub>4</sub>, 25 µg/l thiamine-HCl, 100 mg/l Yeast Extract, 100 mg/l sodium deoxycholate, pH 5.5, 100 ~~mg/L~~ mg/l cefotaxime, 100 ~~mg/L~~ mg/l hygromycine, 18 g/l agar). The transfer of spores to fresh selection plates was repeated three times. In this way, the transformant *Blakeslea trispora* GMO 3005 was isolated. Alternatively, the GMO (genetically modified organisms) were selected by applying the spores individually to CM-17 agar containing 100 mg/l cefotaxime, 100 mg/l hygromycin, by means of the BectonDickinson FacsVantage+Diva Option. In this case, fungal mycelium formed only where the spores had been genetically modified.

In the specification at page 41, line 22, please replace the paragraph which starts with "200 ml of MEP" with the following amended paragraph:

200 ml of MEP medium (30 g/l malt extract, 3 g/l pepton e, pH 5.5) were inoculated with  $10^5$  to  $10^7$  spores of the *Blakeslea trispora* GMO 3005 transformant and incubated on a rotary shaker at 200 rpm and 26°C for 7 days. To detect successful transformation, DNA was isolated from the mycelium (Peqlab Fungal DNA Mini Kit) and used in a PCR (program: 94°C for 1 min, then 30 cycles of 1 min. at 94°C, 1 min. at 58°C, 1 min. at 72°C, ~~each~~).

In the specification at page 43, line 22, please replace the paragraph which starts with “A small portion” with the following amended paragraph:

A small proportion of the spores of *Blakeslea trispora* or of the genetically modified *Blakeslea trispora* strains is by nature mononuclear. To produce homonuclear recombinant strains comprising the foreign DNA of pBinAHyg or pBinAHyg derivatives, the mononuclear spores were sorted out by means of FACS and plated on MEP (30 g/l malt extract, 3 g/l peptone, pH 5.5, 18 g/l agar) containing 100 mg/l cefotaxime and 100 mg/l hygromycin. The mycelia produced here were homonuclear. For sorting FACS, the spores of a 3 day old smear were washed off with 10 ml of Tris-HCl 50 mMol + 0.1% Span20 per agar plate. The spore concentration was from  $0.5$  to  $0.8 \times 10^7$  spores per ml. 1 ml of DMSO and 10 µl of Syto 11 (dye stock solution in DMSO, Molecular Probes No. S-7573) were added to 9 ml of spore suspension. This was followed by staining at 30°C for 2 h. Selection and application were carried out by means of a Becton Dickinson FacsVantage+Diva Option type instrument. First, a size selection was carried out in order to separate individual spores from aggregates and contaminations. These spores were then applied sorted according to their fluorescence (excitation = 488 nm; emission = 530 nm). The left shoulder of the Gauss curve of the fluorescence frequency distribution contained the mononuclear spores.

In the specification at page 50, line 14, please replace the section which starts with “p-carRA-HPcrtZ-pcarRA-NPcrtW” with the following amended section:

- p-carRA-HPcrtZ-pcarRA-NPcrtW, comprising the gene of the HPcrtZ hydroxylase from *Haematococcus pluvialis* Flotow NIES-144 and the gene of the NPcrtW ketolase from *Nostoc punctiforme* PCC73102 (ORF148, Accession No. ~~NZ-AABC01000196~~

NZ\_AABC01000196), both in each case under the control of the *Blakeslea trispora* pcarRA promoter (Seq. pBinAHygBTpcarRA-HPcrtZ-BTpcarRA-NpucrtW, SEQ ID NO:50, Fig. 18);

In the specification at page 52, line 25, please replace the paragraph which starts with “This DNA probe” with the following amended paragraph:

This DNA probe was used for ~~serreeing~~ screening the cosmid gene library. A clone whose cosmid hybridized with said DNA probe was identified. The insert of this cosmid was sequenced. The DNA sequence comprised a section which was assigned to the gene of an MHG-CoA reductase [SEQ ID NO 75].

In the specification at page 65, line 10, please replace the paragraph which starts with “In the preferred embodiment” with the following amended paragraph:

Extracts of the fermentation ~~breth~~ broths were used as matrix. Prior to HPLC, each sample was filtered through a 0.22  $\mu$ m filter. The samples were kept cool and protected from light. In each case 10 mg were weighed and dissolved in 100 ml of THF for calibration. The following carotenoids with the following retention times were used as standard:  $\beta$ -carotene (12.5 min), lycopene (11.7 min), echinenone (10.9 min), cryptoxanthin (10.5 min), canthaxanthin (8.7 min), zeaxanthin (7.6 min) and astaxanthin (6.4 min) [see Fig. 23].